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Enhanced Removal of a Human Norovirus Surrogate from Fresh Vegetables and Fruits by a Combination of Surfactants and Sanitizers[▽]

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Fruits and vegetables are major vehicles for transmission of food-borne enteric viruses since they are easily contaminated at pre- and postharvest stages and they undergo little or no processing. However, commonly used sanitizers are relatively ineffective for removing human norovirus surrogates from fresh produce. In this study, we systematically evaluated the effectiveness of surfactants on removal of a human norovirus surrogate, murine norovirus 1 (MNV-1), from fresh produce. We showed that a panel of surfactants, including sodium dodecyl sulfate (SDS), Nonidet P-40 (NP-40), Triton X-100, and polysorbates, significantly enhanced the removal of viruses from fresh fruits and vegetables. While tap water alone and chlorine solution (200 ppm) gave only <1.2-log reductions in virus titer in all fresh produce, a solution containing 50 ppm of surfactant was able to achieve a 3-log reduction in virus titer in strawberries and an approximately 2-log reduction in virus titer in lettuce, cabbage, and raspberries. Moreover, a reduction of approximately 3 logs was observed in all the tested fresh produce after sanitization with a solution containing a combination of 50 ppm of each surfactant and 200 ppm of chlorine. Taken together, our results demonstrate that the combination of a surfactant with a commonly used sanitizer enhanced the efficiency in removing viruses from fresh produce by approximately 100 times. Since SDS is an FDA-approved food additive and polysorbates are recognized by the FDA as GRAS (generally recognized as safe) products, implementation of this novel sanitization strategy would be a feasible approach for efficient reduction of the virus load in fresh produce.

Viruses cause more than 67% of all food-borne illnesses worldwide (21, 29, 36). Human norovirus is a major enteric food-borne virus that is a significant problem in foods due to its small infectious dose (<10 particles) and its high stability in the environment (20, 43, 47). It is estimated that at least 90% of acute nonbacterial gastroenteritis outbreaks can be attributed to norovirus infection, but this number may even be underestimated due to the large number of unreported infections and the lack of methods for rapid detection of the virus (17, 21, 29, 47). According to a recent report from the Centers for Disease Control and Prevention, approximately 48 million people suffer from norovirus-induced gastroenteritis each year in the United States, there are 128,000 hospitalizations, and 3,000 people die from norovirus each year (12). Outbreaks of human norovirus are common in any environment where people are in close contact, such as cruise ships, restaurants, hotels, schools, the military, nursing homes, and hospitals (1, 17, 21, 26, 36, 47). Transmission of norovirus is primarily by the fecal-oral route, either by person-to-person spread or by ingestion of contaminated food or water (13, 21, 27, 39, 42). The primary symptoms of human norovirus infection include diarrhea, vomiting, fever, chills, and extreme dehydration. It has been a challenge to work with human norovirus since it does not propagate in cell culture and there is no suitable animal model for the virus (19, 53). For this reason, studies of human norovirus must rely on surrogates such as murine norovirus 1 (MNV-1) or feline calicivirus (FCV) (2, 7, 11, 53). Because of these challenges, human norovirus and other caliciviruses are classified as category B priority biodefense agents according to the National Institute of Allergy and Infectious Diseases (NIAID).

Fresh produce is at a high risk for contamination by norovirus because it normally undergoes little or no processing and can be contaminated at any step from preharvest to postharvest. According to recent outbreak data, fruits and vegetables are major vehicles in the transmission of food-borne illness (8, 9, 18, 24, 34). It has been reported that norovirus accounted for more than 40% of outbreaks in fresh produce from 1998 to 2005 in the United States (18). These outbreaks of norovirus have occurred in lettuce, tomatoes, melons, strawberries, raspberries, fresh cut fruits, and other vegetables (10, 18, 24, 34, 40, 51). One major route with a high probability of contamination is the use of contaminated water for irrigation or washing. Contamination may also be caused by infected workers handling the food during harvesting, processing, or distribution (10, 16, 18, 24, 34). With an increasing number of people striving to eat healthier by increasing their consumption of fruits and vegetables, this has become a major public health concern (4, 5, 18, 24, 44, 45). However, while numerous studies of bacterial contamination of fresh produce to have been reported date, knowledge about viral contamination of fresh produce remains limited.

In current industry, fresh produce usually undergoes a brief

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sanitization step after harvest from the field. Unfortunately, current commonly used sanitizers are relatively not effective in removing viral contaminants from fresh produce (2, 3, 5, 15, 22). The most common sanitizer, a solution containing 200 ppm of chlorine, typically only gives a <1.2-log virus reduction in fresh produce (3, 4, 18, 22, 43). Recently, Baert et al. (2009) found that tap water washing only gave an average reduction of 0.94 logs in shredded lettuce, while the addition of 200 ppm of sodium hypochlorite only led to an additional 0.48 logs, and the addition of 80 ppm of peroxyacetic acid brought about a reduction of only 0.77 additional logs (3). Therefore, there is an urgent need to develop a more effective sanitizer for removal of noroviruses from fresh produce.

Surfactants are surface-active compounds that can reduce the surface tension of a liquid. The addition of surfactants in a washing procedure will make the liquid spread more easily and lower the interfacial tension between the two liquids or between a liquid and a solid. In addition, they may act as detergents, wetting agents, emulsifiers, foaming agents, and dispersants (6, 37). Surfactants contain both a hydrophilic group and a hydrophobic group, which interact with the substance they are mixed with in order to alter the surface properties of the water either at the water-and-air interface or at the water-and-solid interface (6, 37). Because of these properties, surfactants allow the release of tightly bound contaminations such as food-borne pathogens from the surface, which leads us to hypothesize that surfactants may enhance the removal of food-borne pathogens from fresh produce. Out of numerous ionic (anionic or cationic) and nonionic surfactants, we chose sodium dodecyl sulfate (SDS), polysorbates (such as Tween 20, Tween 65, and Tween 80), Triton X-100, and NP-40 for the following reasons: (i) SDS is an anionic surfactant and an FDA-approved food additive (FDA 21 CFR 172.822), (ii) polysorbates are a class of nonionic surfactants and GRAS (generally recognized as safe) substances recognized by the FDA (21 CFR 172.840, 172.836, and 172.838), and (iii) Triton X-100 and NP-40 are two other widely used nonionic surfactants that may have similar effects in enhancing sanitization, although there is no record as to the safety of these two surfactants in the FDA Code of Federal Regulations to date.

Here, we report a systematic evaluation of the effectiveness of surfactants in removal of viruses from fresh produce, using murine norovirus 1 (MNV-1) as a surrogate. We found that surfactants alone or a combination of surfactants (at low concentrations) with chlorine solution significantly enhanced the removal of MNV-1 from fresh produce. Using this strategy, >3-log reductions in virus titer were achieved in either fruits (strawberries and raspberries) or leafy greens (cabbage and lettuce). These results strongly support the idea that the combination of a surfactant and chlorine solution is a novel and feasible approach for enhancing the safety of fresh produce.

MATERIALS AND METHODS

Cell culture and virus stock. Murine norovirus strain MNV-1 was a generous gift from Herbert W. Virgin IV, Washington University School of Medicine (28). MNV-1 was propagated in murine macrophage cell line RAW 264.7 (ATCC, Manassas, VA) as follows. RAW 264.7 cells were cultured and maintained in Dulbecco's modified Eagle medium (DMEM; Invitrogen, Carlsbad, CA) with the addition of 10% fetal bovine serum (FBS; Invitrogen) at 37°C under a 5% CO_2 atmosphere. To prepare MNV-1 stock, confluent RAW 264.7 cells were infected with MNV-1 at a multiplicity of infection (MOI) of 20. After 1 h of incubation at 37°C, 15 ml DMEM supplemented with 2% FBS was added. After 2 days postinfection, the virus was harvested by freeze-thawing three times, and the supernatant was collected after centrifugation at 5,000 × *g* for 20 min at 4°C.

The vesicular stomatitis virus (VSV) Indiana strain was generously provided by Sean Whelan at Harvard Medical School (32). VSV was grown in baby hamster kidney (BHK-21) cells (ATCC, Manassas, VA). VSV stock was prepared as previously described (32). Briefly, confluent BHK-21 cells were infected with VSV at a MOI of 3. After 1 h of incubation at 37°C, 15 ml of DMEM supplemented with 2% FBS was added. Virus was harvested after 18 h postinoculation by centrifugation at 5,000 × *g* for 10 min at 4°C. The virus suspension was stored at −80°C in aliquots.

MNV-1 and VSV plaque assay. MNV-1 plaque assay was performed with RAW 264.7 cells as described previously (28). In brief, RAW 264.7 cells were seeded in 6-well plates (Corning Life Sciences, Wilkes-Barre, PA) at a density of 2×10^5 cells per well. After 24 h of incubation, cells were infected with 400 μl from a 10-fold dilution scheme of the virus. After 1 h of incubation at 37°C with agitation every 15 min, the cells were overlaid with 2.5 ml of minimum Eagle medium (MEM) containing 2% FBS, 1% sodium bicarbonate, 0.1 mg/ml of kanamycin, 0.05 mg/ml of gentamicin, 15 mM HEPES (pH 7.7), 2 mM L-glutamine, and 1% agarose. After incubation at 37°C for 2 days, the plates were fixed with 10% formaldehyde, and the plaques were then visualized by staining with crystal violet. A VSV plaque assay was performed in the same way except that Vero cells were used in the assays and the plaques were fixed at 24 h postinoculation (32, 35).

Inoculation of fresh produce with MNV-1. Fresh produce samples (strawberries, raspberries, cabbage, and romaine lettuce) were purchased from a local supermarket. A sample consisted of 50 g placed in a sterile plastic bag. MNV-1 stock (5.0×10^8 PFU/ml) was added to each sample to reach an inoculation level of 3.0×10^6 PFU/g. The bag was heat sealed using an AIE-200 Impulse sealer (American International Electric, Whittier, CA), and the samples were mixed thoroughly by shaking them at a speed of 200 rpm at room temperature for 1 h to allow attachment of virus to the sample.

Sanitization procedure. SDS (powder), NP-40, Triton X-100, and Tween 20 (liquid) were purchased from Sigma (St. Louis, MO), and chlorine bleach containing 6% sodium hypochlorite was purchased from a local supermarket. The MNV-1-inoculated fresh produce was sanitized by tap water, a solution containing 200 ppm of chlorine, surfactant alone, and solutions containing both surfactants and chlorine. For strawberries and raspberries (50 g), the amount of washing solution was 2 liters. For lettuce and cabbage (50g), 4 liters of washing solution was used. Freshly prepared washing solution was used for every replication, and the washing container was cleaned and rinsed out between replications. Each sample was washed by each sanitizer with gentle agitation for 2 min. After sanitization, the fresh produce was drained and placed into a stomacher bag. The remaining viruses were eluted by addition of 20 ml of phosphate-buffered saline (PBS) solution and stomached for 3 min. To neutralize any potential residual chlorine, 100 μl of 0.25 M sodium thiosulfate was added to each sample. The residual detergents were removed by a Detergent-OUT Micro kit (Millipore, Billerica, MA). The viral survivors were determined by a plaque assay.

Virucidal assay. A nonenveloped virus (MNV-1) and an enveloped virus (VSV) were used to test whether surfactants can directly inactivate the viruses. Nine-hundred-microliter volumes of MNV-1 (10^8 PFU/ml) and VSV (10^{10} PFU/ml) stocks were incubated with 100 μl of each surfactant at the desired concentration at either 25 or 37°C. At each time point, 50 μl of the virus sample was collected, and the detergents were removed by a Detergent-OUT Micro kit (Millipore). The virus survivors were determined by a plaque assay. To avoid any cytotoxic effect that may be caused by surfactants, the inoculum solutions were removed after 1 h of incubation before the overlay was added. For VSV inactivation, only one concentration (200 ppm) of each surfactant was used. The virus samples were collected after 1, 4, 8, 12, 24, 36, and 48 h of incubation. For MNV-1 inactivation, four concentrations (50, 200, 1,000, and 10,000 ppm) of each surfactant were used. The time points were 1, 4, 8, 12, 24, 36, 48, 60, and 72 h. The kinetics of viral inactivation were generated for each surfactant.

Purification of MNV-1 and VSV. To grow a large stock of MNV-1, 18 confluent T150 flasks of RAW 267.1 cells were infected with MNV-1 at a MOI of 20 in a volume of 3 ml of DMEM. At 1 h postadsorption, 15 ml of DMEM with 2% FBS was added to the flasks, and infected cells were incubated at 37°C for 48 h. When an extensive cytopathic effect (CPE) was observed, cell culture fluid was harvested and subjected to three freeze-thaw cycles to release virus particles. The purification of MNV-1 was performed using the method described by Katpally et al. (2008), with minor modifications (29). Briefly, virus suspension was centrifuged at 10,000 × *g* for 15 min to remove cellular debris. The supernatant was digested with DNase I (10 μg/ml) and $MgCl_2$ (5 mM) at room temperature. After

1 h of incubation, 10 mM EDTA and 1% lauryl sarcosine were added to stop nuclease activity. Virus was concentrated by centrifugation at 82,000 × *g* for 6 h at 4°C in a Ty 50.2 rotor (Beckman). The pellet was resuspended in PBS and further purified by centrifugation at 175,000 × *g* for 6 h at 4°C through a sucrose gradient (7.5 to 45%) in an SW55 Ti rotor (Beckman). The final virus-containing pellets were resuspended in 100 μl PBS. The virus titer was determined by a plaque assay with RAW 264.7 cells. Viral protein was measured by Bradford reagent (Sigma Chemical Co., St. Louis, MO).

Purification of VSV was performed by the method described in our previous publication (32, 35). Briefly, 10 confluent T150 flask BHK-21 cells were infected by VSV at a MOI of 0.01. At 1 h postadsorption, 15 ml of DMEM (supplemented with 2% FBS) was added to the cultures, and infected cells were incubated at 37°C. After 24 h postinfection, cell culture fluid was harvested by centrifugation at 3,000 × *g* for 5 min. Virus was concentrated by centrifugation at 40,000 × *g* for 90 min at 4°C in a Ty 50.2 rotor. The pellet was resuspended in NTE buffer (100 mM NaCl, 10 mM Tris, 1 mM EDTA [pH 7.4]) and further purified through 10% sucrose NTE by centrifugation at 150,000 × *g* for 1 h at 4°C in an SW50.1 rotor. The final pellet was resuspended in 0.3 ml of NTE buffer. The virus titer was determined by a plaque assay with Vero cells, and the protein content was measured by Bradford reagent (Sigma Chemical Co., St. Louis, MO).

Transmission electron microscopy. Negative staining electron microscopy of purified virions was performed to determine whether chlorine and surfactants damage the virus particles. Sixty microliters of highly purified MNV-1 and VSV suspension was incubated with chlorine (200 ppm), SDS (200 or 10,000 ppm), or a combination of chlorine (200 ppm) and SDS (200 ppm) at 37°C for 48 h. A viral plaque assay was conducted to confirm the inactivation of virus. Twenty-microliter aliquots of either treated or untreated samples were fixed in copper grids (Electron Microscopy Sciences, Hatfield, PA) and negatively stained with 1% ammonium molybdate. Virus particles were visualized by an FEI Tecnai G2 Spirit transmission electron microscope (TEM) at 80 kV at the Microscopy and Imaging Facility at The Ohio State University. Images were captured on a MegaView III side-mounted charge-coupled-device (CCD) camera (Soft Imaging System, Lakewood, CO), and figures were processed using Adobe Photoshop software (Adobe Systems, San Jose, CA).

Statistical analysis. All experiments were done in triplicate. The surviving viruses were expressed as mean log viral titer ± standard deviation. Statistical analysis was done using one-way analysis of variance (ANOVA), with a *P* value of <0.05 being statistically significant.

RESULTS

Enhanced removal of a human norovirus surrogate from fresh strawberries by SDS alone or by combination of SDS with chlorine solution. It has been well documented that traditional sanitizers such as a solution containing 200 ppm of chlorine are relatively not effective in removal of viruses from fresh produce (3, 4, 18, 22). Based on the fact that surfactants can reduce the surface tension of liquids and possibly possess virucidal activities, we hypothesized that surfactants may enhance the removal of virus from fresh produce. To address this premise, we first evaluated the reduction of MNV-1 on strawberries using either SDS alone or a combination of SDS with a solution containing 200 ppm of chlorine. As described in Materials and Methods, MNV-1-contaminated samples (50 g of strawberries) were washed with either SDS solution alone or combination of SDS with chlorine solution for 2 min at room temperature. The amount of surviving viruses after treatment was quantified by a plaque assay. Figure 1 (gray bars) shows the viral survivors after each treatment. Consistent with previous observations, tap water washing only gave a 0.8-log reduction in virus titer. A solution with 200 ppm of chlorine brought about a slight increase in virus reduction (1.0 log) in comparison with tap water alone, but such increase was not statistically significant ($P > 0.05$). Interestingly, the virus removal was significantly improved upon sanitization with an SDS solution. Moreover, the amount of reduction in MNV-1 titer gradually increased as the concentration of SDS increased. For example,

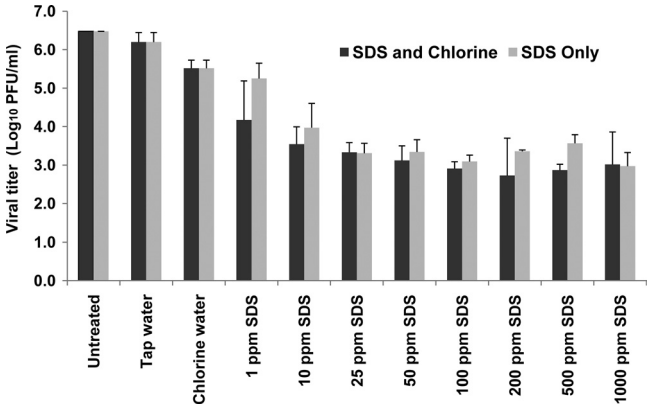

FIG. 1. Effect of SDS concentration on removal of MNV-1 from strawberries. Fresh strawberries were inoculated with MNV-1 to give a final concentration of approximately 3×10^6 PFU/g. After incubation for 1 h, the samples were sanitized with gentle agitation for 2 min in washing solutions containing various levels of SDS alone (gray bars) or 200 ppm of sodium hypochlorite in combination with various levels of SDS (black bars). After sanitization, the fresh produce was stomached, and the surviving viruses were quantified by a plaque assay. Data are the means of results from three replicates. Error bars represent 1 standard deviation.

a 3.14-log reduction in virus titer was achieved when a solution with 50 ppm of SDS was used, indicating that 50 ppm of SDS is significantly more efficient than tap water and a solution with 200 ppm of chlorine ($P < 0.05$). However, 1 and 10 ppm of SDS gave about 1.25- and 2.60-log reductions in virus titer, respectively. A 100-ppm concentration of SDS slightly increased virus reduction (3.41 log) compared to a 50-ppm concentration. However, the washing efficiency (log reduction in virus titer) of SDS did not continuously increase after its concentration reached 200 ppm. For example, 1,000 ppm of SDS gave a 3.51-log reduction in virus titer, which was only slightly higher than a 200-ppm concentration (3.12-log reduction) ($P > 0.05$). Overall, these results demonstrated that SDS solution alone significantly increased the removal of virus from strawberries even at a very low concentration (20 to 100 ppm).

We then attempted to achieve improved virus reduction by combining SDS and chlorine solution. With the use of an identical washing procedure, strawberries were washed with chlorine solution containing increasing amounts of SDS ranging from 10 to 1,000 ppm. As shown in Fig. 1 (black bars), SDS enhanced the efficiency of virus removal in a concentration-dependent manner. While chlorine solution alone only gave a 0.96-log reduction in virus titer, a 2.94-log reduction in virus titer was observed when a very small amount of SDS (10 ppm) was added to the chlorine solution. Notably, a 3.36-log reduction in virus titer was achieved using a chlorine solution containing 50 ppm of SDS. Similar to what was observed for SDS solution alone, the washing efficiency was not further enhanced by addition of SDS into chlorine solution at 200-ppm or higher concentrations. Apparently, these observations indicate that virus removal was significantly enhanced by combination of SDS with chlorine solution.

We noticed that there is no significant difference in virus reduction between SDS solution and SDS-chlorine combined solution. For example, the combination of 50 ppm of SDS and

a solution containing 200 ppm of chlorine led to a reduction in virus titer of 3.36 logs, which is just slightly higher than the reduction caused by 50 ppm of SDS alone (3.14 logs). Further comparisons of the washing efficiencies of SDS and SDS-chlorine solutions showed that SDS and SDS-chlorine solutions have comparable efficacies in removing MNV-1 from strawberries (data not shown). There may be a minimal amount of chlorine and detergents remaining in fresh produce after sanitization, which may interfere with the viral plaque assay. To minimize this effect, the residual chlorine was neutralized by sodium thiosulfate, and detergents were removed by a Detergent-Out kit. Actually, our results showed that these residual chlorine and detergents had a negligible effect on the viral plaque assay (data not shown). Taken together, these results clearly demonstrated that virus removal from strawberries is significantly improved by using SDS alone or a combination of SDS and chlorine solution. Under our experimental condition, we also concluded that 50 ppm of SDS is an optimal working concentration since it is cost-effective, highly efficient in virus removal, and safe to consumers. To our knowledge, this is the first report showing that a sanitizer is able to achieve a viral reduction of more than 3 logs in fresh produce. Apparently, SDS solution alone or the combination of SDS and chlorine enhanced the efficiency of virus removal by over 100 times in comparison with traditional sanitizer only (such as chlorine).

Enhanced removal of a human norovirus surrogate from other fruits and vegetables by SDS solution or by a combination of SDS and chlorine solution. Our ultimate goal is to develop a novel sanitizer that can be used to enhance the safety of fruits and vegetables. Hence, after it was observed that SDS was able to give a significantly higher virus reduction in strawberries, we expanded our studies to other fruits and vegetables. We selected two leafy greens (cabbage and romaine lettuce) and one other fruit (raspberries) since they are often contaminated by norovirus and the surfaces of these products are strikingly different from that of strawberries. Like strawberries, MNV-1-contaminated cabbage, lettuce, and raspberry samples were washed with tap water, chlorine, SDS (50 ppm), and SDS (50 ppm)-chlorine solutions, and the surviving viruses were quantified by a plaque assay. Similar to our previous observation for strawberries, the tap water and 200-ppm-chlorine solution only brought about 1.23- and 1.48-log reductions in virus titer in raspberries, respectively (Fig. 2). Surprisingly, 50 ppm of SDS alone caused a 2.63-log reduction in virus titer in raspberries (Fig. 2), in comparison with a 3.14-log reduction in virus titer in strawberries (Fig. 1). Moreover, a 3.05-log reduction in virus titer was achieved when SDS (50 ppm) was combined with chlorine solution (Fig. 2). In cabbage, tap water and chlorine solution gave 0.61- and 1.31-log reductions in virus titer, respectively (Fig. 2), whereas SDS alone (50 ppm) exhibited an efficiency of virus reduction virtually equivalent to that of chlorine solution. Importantly, a 2.56-log reduction in virus titer was obtained when SDS (50 ppm) was combined with chlorine solution (Fig. 2). For lettuce samples, tap water and chlorine solution only led to 0.23- and 1.12-log reductions in virus titer, respectively (Fig. 2). In contrast, SDS alone (50 ppm) gave a 2.26-log reduction in virus titer, which is significantly higher than that of chlorine solution ($P < 0.05$). In the meanwhile, combination of SDS and chlorine further enhanced the virus removal (2.90-log reduction in virus titer).

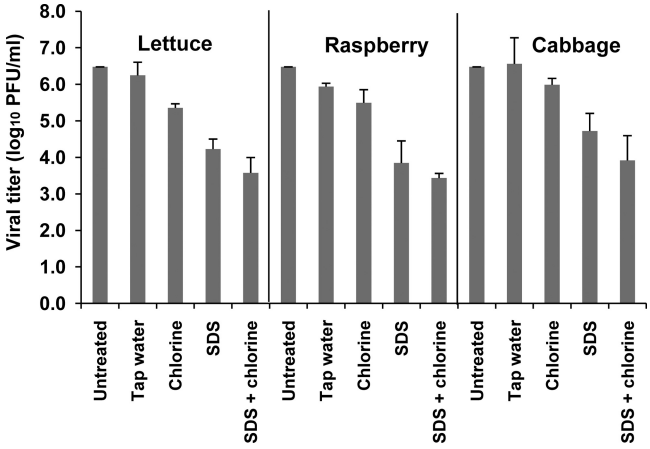

FIG. 2. Enhanced removal of MNV-1 from lettuce, cabbages, and raspberries by SDS solution or by a solution containing a combination of SDS with chlorine. Fresh lettuce, cabbage, or raspberries were inoculated with MNV-1 to give a final concentration of approximately 3×10^6 PFU/g. After 1 h of incubation, the samples were sanitized in washing solutions containing 50 ppm of SDS alone and also in combination with 200 ppm of sodium hypochlorite. Data are the means of results from three replicates. Error bars represent 1 standard deviation.

Therefore, our results demonstrated that the combination of SDS (50 ppm) and chlorine (200 ppm) brought about the biggest reduction in virus titer, at about 3 logs, for all four tested fruits or vegetables. In addition, while SDS solution alone generally improved the sanitization efficiency compared to chlorine solution, there were notable differences in virus reduction among different types of produce. For example, SDS alone at the concentration of 50 ppm was able to efficiently remove the viruses from strawberries, with a 3.14-log reduction in virus titer, whereas the corresponding virus reductions in raspberries, cabbage, and lettuce were 2.63, 1.80, and 2.26 log, respectively. Taken together, these results demonstrate that the addition of SDS in chlorine solution significantly enhances the removal of virus from fruits and vegetables in general.

Enhanced removal of viruses from fruits and vegetables by other surfactants. Since SDS exhibited significant potential in enhancing virus removal from fresh produce, we continued to determine whether other surfactants retain similar potentials in enhancing the removal of norovirus from produce. These commonly used surfactants include NP-40, Triton X-100, and polysorbates (such as Tween 20, Tween 65, and Tween 80). The experimental design and sanitization procedure for each surfactant were essentially identical to that with SDS. Four types of MNV-1-contaminated fresh produce (strawberries, raspberries, cabbage, and lettuce) were washed by tap water, chlorine (200 ppm), surfactant (50 ppm), and surfactant (50 ppm)-chlorine (200 ppm) solutions for 2 min at room temperature. Tap water and chlorine solutions did not give virus reductions of more than 1 log in any tested fruit or vegetable (Fig. 3). While NP-40 alone (50 ppm) gave more virus reduction (1.2 to 2.7 log) than tap water or chlorine solution, the combination of NP-40 (50 ppm) and chlorine (200 ppm) was most efficient at removing norovirus from all tested samples, as evidenced by the fact that it gave virus reductions of about 3.0 logs for raspberries, lettuce, and cabbage and up to 3.5 logs for

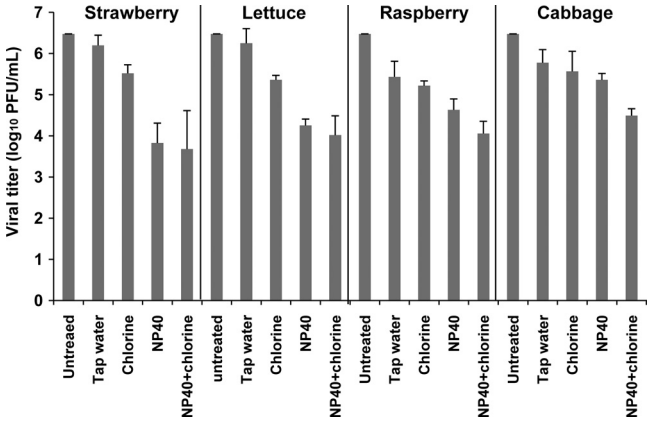

FIG. 3. Enhanced removal of MNV-1 from fruits and vegetables by NP-40. Fresh produce samples were inoculated with MNV-1 to give a final concentration of approximately 3×10^6 PFU/g. After 1 h of incubation, the samples were washed with tap water or a solution containing 200 ppm of chlorine, 50 ppm NP-40, or 50 ppm of NP-40 in combination with 200 ppm of sodium hypochlorite. The surviving viruses after washing were quantified by a plaque assay. Data are the means of results from three replicates. Error bars represent 1 standard deviation.

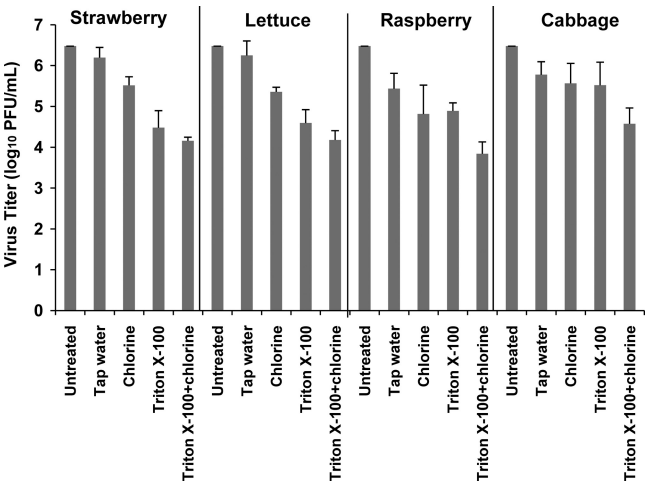

FIG. 5. Enhanced removal of MNV-1 from fruits and vegetables by Triton X-100. Fresh produce samples were inoculated with MNV-1 to give a final concentration of approximately 3×10^6 PFU/g. After 1 h of incubation, the samples were washed with tap water or a solution containing 200 ppm of chlorine, 50 ppm of Triton X-100, or 50 ppm of Triton X-100 in combination with 200 ppm of sodium hypochlorite. The surviving viruses after washing were quantified by a plaque assay. Data are the means of results from three replicates. Error bars represent 1 standard deviation.

strawberries (Fig. 3). Similar results were observed when Tween 20 was used for sanitization (Fig. 4), which showed that the combination of Tween 20 and chlorine was the most effective strategy for removing viruses (reduction of approximately 3 logs). Subsequently, we also tested other polysorbates, such as Tween 80 and Tween 65. Similar to Tween 20, both Tween 80 and Tween 65 significantly enhanced virus removal (3- to 3.6-log reduction in virus titer) from all the tested fresh produce (data not shown). Interestingly, results from Triton X-100 (as well as SDS) are somehow different. For raspberries and cabbage, Triton X-100 (50 ppm) gave results similar to those given by chlorine (200 ppm), but for strawberries and romaine lettuce, Triton X-100 (50 ppm) caused almost a 1-log additional reduction compared to the chlorine solution (Fig. 5). In any case, the combination of Triton X-100 and chlorine remained the most efficient sanitizer for removing MNV-1 from fresh produce (approximately 3-log reduction in virus titer). Taken together, these results demonstrate that surfactants other than SDS also significantly enhanced virus removal from fresh produce and that the combination of a surfactant and chlorine was the most effective sanitizer.

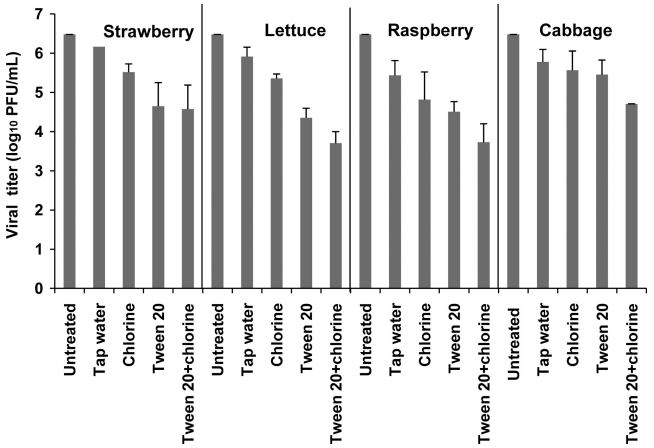

FIG. 4. Enhanced removal of MNV-1 from fruits and vegetables by Tween 20. Fresh produce samples were inoculated with MNV-1 to give a final concentration of approximately 3×10^6 PFU/g. After 1 h of incubation, the samples were washed with tap water or a solution containing 200 ppm of chlorine, 50 ppm of Tween 20, or 50 ppm of Tween 20 in combination with 200 ppm of sodium hypochlorite. The surviving viruses after washing were quantified by a plaque assay. Data are the means of results from three replicates. Error bars represent 1 standard deviation.

Viral inactivation by surfactants. Since all tested surfactants enhanced the removal of MNV-1 from fresh produce, one may argue that surfactants can directly inactivate the virus during sanitization. To address this argument, we investigated the virucidal activities of surfactants by directly adding the surfactants to virus stock. Briefly, after incubation of MNV-1 with each surfactant, virus samples were collected after certain time points of incubation, residual detergents were removed, and virus survivors were determined by a plaque assay. As shown in Fig. 6, all four surfactants showed virucidal activity against MNV-1. Viral titer gradually reduced when incubation time increased. There was no significant difference in virus reduction among these four surfactants at the concentrations of 50 and 200 ppm ($P > 0.05$) (Fig. 6A and B). At 72 h of incubation time, approximately 2.0- to 2.5-log reductions in virus titer were observed for all four surfactants. At 1,000 ppm, SDS was the most effective surfactant, giving the highest reduction in MNV-1 titer after 72 h of incubation (Fig. 6C). Virucidal activity of SDS dramatically increased when the concentration was increased to 10,000 ppm (Fig. 6D). Interestingly, for NP-40, Triton X-100, and Tween 20, there was no significant increase in virucidal activity at 10,000 ppm compared to the levels for the other three concentrations (50, 200, and 1,000

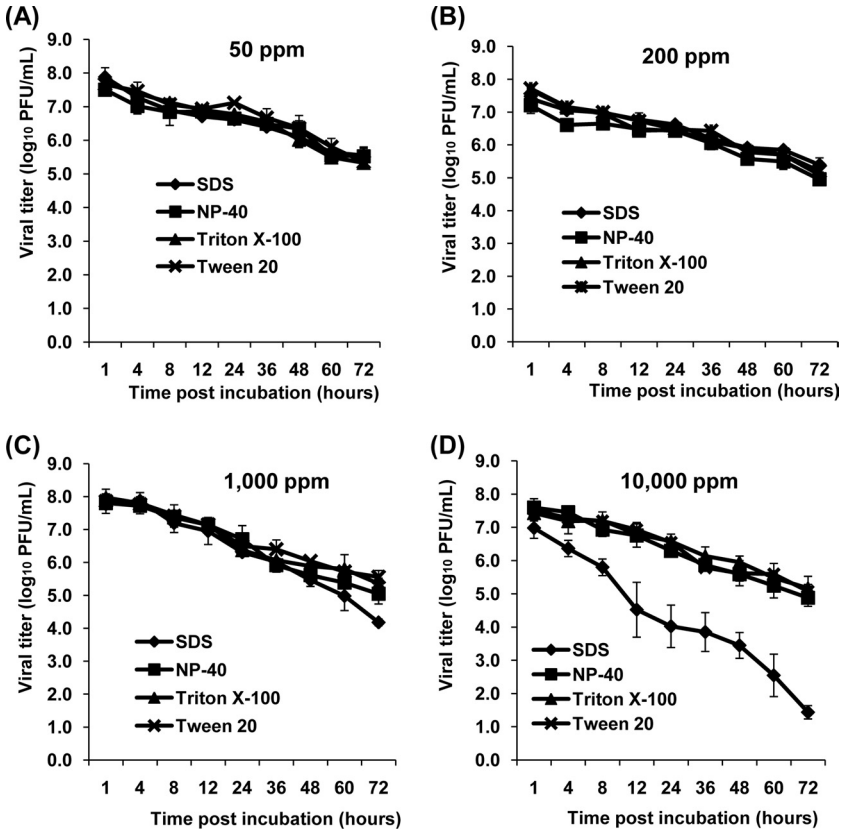

FIG. 6. Inactivation of MNV-1 by surfactants. MNV-1 stock was inoculated with each surfactant (SDS, NP-40, Triton X-100, or Tween 20) and was incubated at 37°C for 72 h. At each time point, 50 μl of virus sample was collected, and detergents were removed by a Detergent-OUT Micro kit. Virus survivors were determined by a plaque assay. Data are the means of results from three replicates. (A) 50 ppm; (B) 200 ppm; (C) 1,000 ppm; and (D) 10,000 ppm.

ppm) ($P > 0.05$). At 72 h of incubation time, 6.1-, 2.4-, 2.5-, and 2.6-log reductions in virus titer were observed for SDS, NP-40, Triton X-100, and Tween 20, respectively. The kinetics of MNV-1 inactivation by Tween 65 and Tween 80 was similar to that for Tween 20 (data not shown). Therefore, SDS appears to have the highest virucidal activity against MNV-1 stock in cell culture medium at 10,000 ppm. It has been shown that 200 ppm of chlorine alone has considerable virucidal activity against MNV-1 (14). Indeed, combination of 200 ppm of SDS and 200 ppm of chlorine significantly enhanced the virucidal activity against MNV-1 (data not shown).

Based on the presence or absence of an envelope, viruses can be classified into enveloped or nonenveloped viruses. The envelopes are typically derived from the host cell membranes (lipids and proteins) and sometimes include viral glycoproteins. In order to further test the virucidal activity, we compared the levels of effectiveness of four surfactants (200 ppm) in the inactivation of VSV, an enveloped virus. We found that VSV is much more sensitive to SDS, NP-40, and Triton X-100 than MNV-1. NP-40 appears to have the highest virucidal activity against VSV, followed by SDS, Triton X-100, and Tween 20. At 72 h of incubation, 10.0-, 7.5-, 6.7-, and 2.7-log reductions in virus titer were observed with NP-40, SDS, Triton X-100, and Tween 20, respectively. Therefore, these results demonstrated that enveloped virus (VSV) is much more sensitive to all surfactants than nonenveloped virus (MNV-1). In addition, SDS is effective in inactivating both MNV-1 and VSV compared to other tested surfactants. For example, 10,000 ppm of SDS dramatically increased virucidal activity against MNV-1 and almost completely inactivated the MNV-1 after 72 h of incubation. For VSV, 200 ppm of SDS gave 7.5-log reductions in virus titer after 72 h of incubation.

Surfactants damage virus particles. To determine how viruses were inactivated by the surfactants, SDS was added to a purified virus stock to give a final concentration of 10,000 ppm, allowed to incubate at 37°C for 72 h, and then negatively stained with ammonium molybdate. As controls, viruses were also incubated with 200 ppm of chlorine and a combination of 200 ppm of chlorine and SDS. Viruses were completely inactivated at this condition, as confirmed a by a plaque assay. The virus particles were visualized by electron microscopy as described previously (33). Figure 7 shows the virus particles in the presence and absence of SDS. MNV-1 is a small round-structured virus of about 30 to 38 nm in diameter (Fig. 7A). After incubation with SDS for 72 h, it can be observed that the outer capsid of the MNV-1 was severely damaged and aggregated (Fig. 7B). The shape of MNV-1 was also altered and was no longer completely circular (Fig. 7B). The virions appeared smaller than 30 nm. We could not find any intact virus particles in samples treated by chlorine (Fig. 7C) or the combination of chlorine and SDS (Fig. 7D). This suggests that the integrity of the viral capsid was completely disrupted by chlorine. For

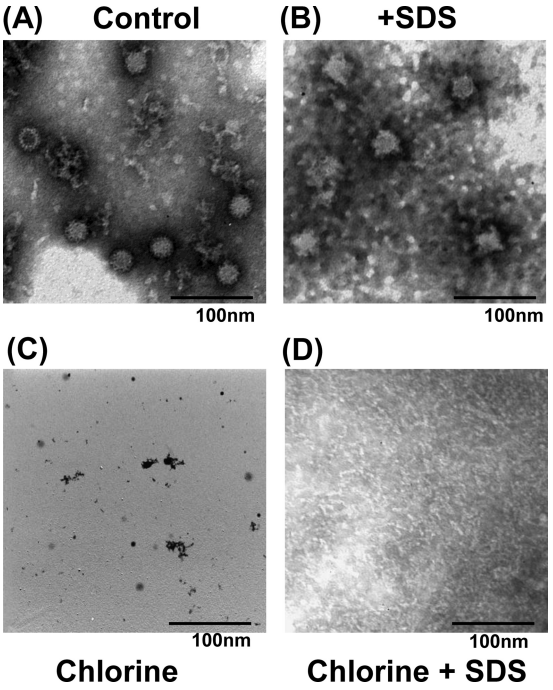

FIG. 7. SDS damages virus particles. Purified MNV-1 was incubated with SDS (10,000 ppm), chlorine (200 ppm), or combination of chlorine (200 ppm) and SDS (200 ppm). Complete virus inactivation was confirmed by a plaque assay. The samples were fixed in copper grids and negatively stained with 1% ammonium molybdate. Virus particles were visualized by transmission electron microscopy. (A) Untreated MNV-1; (B) MNV-1 treated by SDS; (C) MNV-1 treated by chlorine; (D) MNV-1 treated by chlorine and SDS.

VSV, the viral envelope was damaged and the shape was severely distorted by SDS (data not shown). Furthermore, some VSVs were completely disrupted, and genetic materials were spilled out from the particles (data not shown). Therefore, these results indicate that SDS is able to cause significant damages to viral structures of both enveloped and nonenveloped viruses. Similar observations were obtained for other surfactants, NP-40, Triton X-100, Tween 20, Tween 65, and Tween 80 (data not shown).

DISCUSSION

Fresh fruits and vegetables are foods at high risk for norovirus contamination. Currently, no effective methods have been established in reducing virus contaminants in fresh produce. To improve sanitization, we investigated the ability of surfactants to remove a human norovirus surrogate, MNV-1, from a number of fruits and vegetables. We found that virus removal was significantly enhanced by addition of surfactants. We also investigated the virucidal activity of the surfactants when added directly to virus stock in cell culture medium. We found that surfactants had virucidal activity against both enveloped and nonenveloped viruses. Furthermore, we demonstrated that surfactants disrupted viral particles, which resulted in virus inactivation. Our results indicate that surfactants may be a novel and feasible sanitizer for removing viral contaminants from fresh produce, thus reducing the number of produce-related food-borne viral outbreaks.

Surfactants enhance virus removal from fresh produce: factors affecting viral removal. It has been a challenge to remove viruses from fresh produce. Food-borne viruses such as human norovirus are nonenveloped RNA viruses, and their lack of envelope makes them very resistant to agents such as acids, pH changes, environmental stresses, and disinfectants. The typical washing solution used in food industry currently is sodium hypochlorite, but this usually gives only 1 log of virus reduction (3, 4, 18, 22, 44). Surfactants such as SDS, polysorbates, Triton X-100, and NP-40 can reduce the surface tension of water by adsorbing at the liquid-gas or liquid-liquid interface and thus can potentially enhance the removal of viruses from fresh produce. Another alternative is that the surfactants are able to directly denature the virus, resulting in inactivation during sanitization. In the present study, we tested surfactants alone and then in combination with a solution containing 200 ppm of sodium hypochlorite. In all cases, the combination of a tested surfactant and the sanitizer was the most effective way to remove MNV-1 from fresh produce. Also, SDS was the most effective surfactant used, followed by NP-40, Triton X-100, and Tween 20. With the combination of SDS and the chlorine solution, >3-log reductions in virus titer were achieved in all fresh produce samples. Surfactants could be a novel method for enhancing virus removal from fresh produce when combined with a chlorine sanitizer.

There are many factors that can influence the efficiency of virus removal, including choice of a sanitizer, concentration used, washing or contact time, and the nature of the food the virus has attached to. Clearly, the concentration of surfactants plays an important role in virus removal. We found that as the concentration of surfactants increased, the amount of log reduction increased. However, once the concentration of surfactants was over 50 ppm, the virus reduction did not significantly increase. Hence, we decided to use 50 ppm as the primary concentration for all the experiments. While the use of more surfactant led to slightly more reduction in viral titer, it was not enough to outweigh the fact that it would be less cost-effective and potentially cause more health concerns for consumers. A 50-ppm concentration of a surfactant still brought about a significant increase in virus reduction compared to either tap water or chlorine water alone and was most effective in combination with 200 ppm of chlorine.

As for washing contact time and the type of food the virus is attached to, we only tested one time point (2 min) and four types of fresh produce. We expect that there would be a greater log reduction in virus titer if the length of contact time increases. We also expect there should be changes in the amount of reduction when the amount of wash solution is dramatically scaled up as in industry, or if the produce is agitated more aggressively than gently agitating by hand as we did in our study. Foods such as strawberries and raspberries typically showed more reduction than foods such as cabbage and lettuce. This is most likely attributed to the larger surface area that the virus can attach to in the case of cabbage and lettuce. The texture of a strawberry is also much different from that of a piece of lettuce, which may also have an effect on virus attachment and removal ability. In addition, there are many structures in leafy greens such as wrinkles, which may provide shielding effect and thus increase removal difficulty. It has been found that bacterial pathogens become internalized in leafy

greens via stomata where CO_2 and O_2 exchange occurs (18, 33). Recent evidence has suggested that viral pathogens can also be internalized, although it is unknown whether the internalization occurs at the stomata (41, 49, 52). Thus, we cannot exclude the possibility that some viruses already became internalized because we mixed MNV-1 with leafy greens (lettuce and cabbage) for 1 h prior to sanitization. Presumably, fewer viruses can be removed if virus internalization occurs in leafy greens.

Virucidal activity of surfactants. It is well known that surfactants can interact with viral proteins (6, 23, 26, 30, 31). This interaction can influence protein folding/refolding, denaturation, and aggregation. The virucidal activity of surfactants for sexually transmitted viruses has been widely reported. For example, Howett et al. (1998) found that SDS had virucidal activity against papillomaviruses, herpes simplex virus 2 (HSV-2), and human immunodeficiency virus type 1 (HIV-1) (26). Urdaneta and coauthors (2009) found that HIV-1 could be inactivated by SDS in breast milk to avoid transfer of the virus to infants when formula feeding is not practicable (50). Moreover, SDS has been used to prevent the transmission of HIV during sexual intercourse (25). In addition, Song and others (2010) reported that SDS, NP-40, and Triton X-100 were able to reduce the infectivity of hepatitis C virus (46), whereas it has been reported that Triton X-100 was able to partially denature the coat protein of tobacco mosaic virus (TMV) and then induce aggregation of this coat protein (38). However, the effectiveness of surfactants inactivating food-borne viruses is less understood. In this study, we found that all four surfactants were able to inactivate a human norovirus surrogate in a concentration-dependent manner. SDS appears to be the most effective surfactant against MNV-1. Incubation of MNV-1 with a solution containing 200 ppm of SDS at 37°C for 4 h resulted in a 3-log reduction in virus titer. VSV, an enveloped virus, is much more sensitive to surfactants than MNV-1, as evidenced by a 5-log reduction of VSV upon incubation with 200 ppm of SDS at 37°C for 4 h. In our study, we found that the capsid protein of MNV-1 became aggregated after incubation with SDS and that the structure of MNV-1 capsid was severely altered. SDS also disrupted the envelope of VSV and distorted the shape of virions. Taken together, these results suggest that SDS as well as other surfactants can be useful in the inactivation of many viruses, both enveloped and nonenveloped.

Surfactants are a novel intervention for enhancement of the safety of fresh produce. In this study, we were able to show that surfactants are effective in enhancing the removal of a human norovirus surrogate from various types of fresh produce. On one hand, SDS, the most effective surfactant against MNV-1, appears in many daily used products, such as dish soaps, toothpastes, and shampoos, and is an FDA-approved food additive (FDA, 21 CFR 172.822). Shampoos and soaps contain dodecyl sulfate derivatives (sodium or ammonium dodecyl sulfate) at concentrations exceeding 10%. Toothpaste that is routinely used in the oral cavity also has very high concentrations (5 to 8%) of SDS and its derivatives. In foods, SDS is approved for use at concentrations of 25 to 1,000 ppm, depending on the type of products (FDA, 21 CFR 172.822). On the other hand, all of the polysorbates similar in structure to Tween 20 either have GRAS status or are FDA approved food additives as well (FDA, 21 CFR 172.840, 172.836, and 172.838). For example, Tween 80 has been used as an emulsifier in ice cream and custard products, as a dispersing agent in pickle products and gelatin products, as an emulsifier in shortenings and whipped toppings, and as a defoaming agent in the production of cottage cheese (FDA 21 CFR 172.840). Tween 80 is typically used at levels not exceeding 0.1% of the finished product (FDA, 21 CFR 172.840). In addition, even though Triton X-100 and NP-40 are not currently FDA approved, they are similar in function to SDS and Tween 20. Hence, they may be feasible alternatives in the future once more research is conducted on their safety.

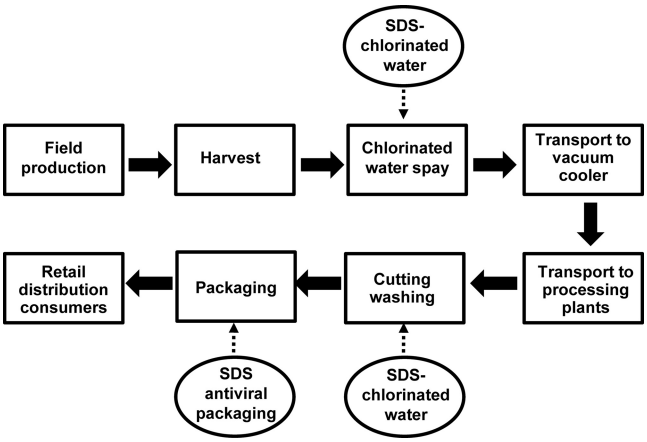

FIG. 8. Potential application of surfactants in minimizing virus contamination in fresh produce. The square boxes show the supply chain flow for leafy greens (such as lettuce) in the fresh produce industry. Proposed interventions for minimizing the virus contamination are shown as ovals.

Figure 8 represents the flow chart of current practice for processing of leafy greens (such as lettuce) in the fresh produce industry. After being harvested from the field, the lettuce is usually subjected to a spray of chlorinated water. To keep fresh, the produce is then transported for vacuum cooling. After the cooling step, the produce continues to be transported to processing plants for cutting, washing by chlorinated water, and packaging, followed by retail distribution. Other produce, such as berries and fruits, may not exactly follow the flow chart shown in Fig. 8. However, these foods are usually washed before or after retail for human consumption. In the chain of this processing event, the use of surfactants could be especially applied during the sanitization step by simply adding a 50-ppm solution of SDS, for example, to the chlorine solution already used currently. Such strategy would yield more virus reduction than what the industry is currently achieving and would not represent much of a change for food-processing companies. To avoid cross contamination, we used fresh washing solution for each sanitization. In industry, the washing solution may be frequently reused. Indeed, we found that viruses were inactivated in chlorine solution or chlorine plus SDS (data not shown). One alternative could be to spray a solution containing surfactants on the fresh produce before the produce is transported to vacuum cooling. Consequently, this spray solution would help with any contamination acquired during preharvest or harvesting, and then the sanitization step with chlorine would be another hurdle for harmful microorganisms. Of note,

a spray solution containing both SDS and chlorine would likely enhance the virucidal activity. Another possible way to use surfactants for enhancing safety would be to coat packages of produce with SDS before they are sent to the stores for consumers to purchase. Since it is known that viruses can survive in foods with high stability for many days to weeks, SDS could kill the viruses on the produce while the produce is stored. Evidently, this approach would only work for types of produce that are packaged instead of free, unpackaged types of produce, but it is still another hurdle technology that could be used in the future. Any or all of these applications could be implemented in the food industry to further enhance the safety of fresh produce and hopefully reduce the incidences of produce-associated outbreaks of norovirus and other types of food-borne viruses or bacteria as well. Further research is needed in this area, but with more studies done on food-borne viruses, this problem can be combated.

Since human norovirus is noncultivable, we are unable to directly address the removal of this biodefense agent from fresh produce. Indeed, most of our understanding of the survival and biology of human norovirus comes from the studies of proper cultivable surrogates such as MNV-1, feline calicivirus (FCV), and canine calicivirus (CaCV) (11, 14, 15, 48, 53). In fact, MNV-1 appears to be the most suitable surrogate because of its stability and genetic relatedness to human norovirus (11, 53). Although it is possible that a different virus may have a different affinity for binding to fresh produce, it is reasonable to propose that the removal of any pathogens from a surface should be increased due to the fact that surfactants can reduce the surface tension. Apparently, this concept has been used in routine products such as soap and toothpaste. Therefore, our study highlights a new notion that combining surfactants and sanitizers may function more efficiently in virus removal from fresh produce. Ongoing studies in our laboratory are for evaluation of other types of commonly used surfactants, such as cationic or zwitterionic detergents or combination of surfactants and organic acids, in removing virus particles in fresh produce.

In conclusion, we found that surfactants can significantly enhance the removal of virus contaminant from fresh produce. To our knowledge, this is the first report of a novel sanitization approach that resulted in a 3-log reduction in virus titer in fresh produce. Implementation of this novel strategy would likely reduce the virus load in fresh produce and improve the safety of fresh produce.

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REFERENCES

1. **Adler, J. L., and R. Zickl.** 1969. Winter vomiting disease. J. Infect. Dis. **119:**668–673.
2. **Bae, J., and K. J. Schwab.** 2008. Evaluation of murine norovirus, feline calicivirus, poliovirus, and MS2 as surrogates for human norovirus in a model of viral persistence in surface water and groundwater. Appl. Environ. Microbiol. 477–484.
3. **Baert, L., et al.** 2009. Efficacy of sodium hypochlorite and peroxyacetic acid to reduce murine norovirus 1, B40-8, *Listeria monocytogenes*, and *Escherichia coli* 0157:H7 on shredded iceberg lettuce and in residual wash water. J. Food Prot. **72:**1047–1054.
4. **Baert, L., J. Debevere, and M. Uyttendaele.** 2009. The efficacy of preservation methods to inactivate foodborne viruses. Int. J. Food Microbiol. **131:** 83–94.
5. **Baert, L., M. Uyttendaele, M. Vermeersch, E. Van Coillie, and J. Debevere.** 2008. Survival and transfer of murine norovirus 1, a surrogate for human noroviruses, during the production process of deep-frozen onions and spinach. J. Food Prot. **71:**1590–1597.
6. **Banat, I. M., R. S. Makkar, and S. S. Cameotra.** 2000. Potential commercial applications of microbial surfactants. Appl. Microbiol. Biotechnol. **53:**495–508.
7. **Belliot, G., A. Lavaux, D. Souihel, D. Agnello, and P. Pothier.** 2008. Use of murine norovirus as a surrogate to evaluate resistance of human norovirus to disinfectants. Appl. Environ. Microbiol. **74:**3315–3318.
8. **Berger, C. N., et al.** 2010. Fresh fruit and vegetables as vehicles for the transmission of human pathogens. Environ. Microbiol. **12:**2385–2397.
9. **Beuchat, L. R.** 1996. Pathogenic microorganisms associated with fresh produce. J. Food Prot. **59:**204–216.
10. **Butot, S., T. Putallaz, R. Amoroso, and G. Sánchez.** 2009. Inactivation of enteric viruses in minimally processed berries and herbs. Appl. Environ. Microbiol. **75:**4155–4161.
11. **Cannon, J. L., et al.** 2006. Surrogates for the study of norovirus stability and inactivation in the environment: a comparison of murine norovirus and feline calicivirus. J. Food Prot. **69:**2761–2765.
12. **Centers for Disease Control and Prevention.** 2010. CDC reports 1 in 6 get sick from foodborne illnesses each year. Centers for Disease Control and Prevention, Atlanta, GA. http://www.cdc.gov/media/pressrel/2010/r101215.html.
13. **Cliver, D. O.** 1997. Virus transmission via food. Food Technol. **51:**71–78.
14. **Cromeans, T. L., A. M. Kahler, and V. R. Hill.** 2010. Inactivation of adenoviruses, enteroviruses, and murine norovirus in water by free chlorine and monochloramine. Appl. Environ. Microbiol. **76:**1028–1033.
15. **Dawson, D. J., A. Paish, L. M. Staffell, I. J. Seymour, and H. Appleton.** 2005. Survival of viruses on fresh produce, using MS2 as a surrogate for norovirus. J. Appl. Microbiol. **98:**203–209.
16. **De Giusti, M., et al.** 2010. The evaluation of the microbial safety of fresh ready-to-eat vegetables produced by different technologies in Italy. J. Appl. Microbiol. **109:**996–1006.
17. **de Wit, M. A. S., et al.** 2007. Large outbreak of norovirus: the baker who should have known better. J. Infect. **55:**188–193.
18. **Doyle, M. P., and M. C. Erickson.** 2008. Summer meeting 2007—the problems with fresh produce: an overview. J. Appl. Microbiol. **105:**317–330.
19. **Duizer, E., et al.** 2004. Laboratory efforts to cultivate noroviruses. J. Gen. Virol. **85:**79–87.
20. **Duizer, E., et al.** 2004. Inactivation of caliciviruses. Appl. Environ. Microbiol. **70:**4538–4543.
21. **Estes, M. K., B. V. Prasad, and R. L. Atmar.** 2006. Noroviruses everywhere: has something changed? Curr. Opin. Infect. Dis. **19:**467–474.
22. **Gulati, B. R., P. B. Allwood, C. W. Hedberg, and S. M. Goyal.** 2001. Efficacy of commonly used disinfectants for the inactivation of Calicivirus on strawberry, lettuce, and a food-contact surface. J. Food Prot. **64:**1430–1434.
23. **Hartmann, S. U., et al.** 2006. Biochemical analysis of human milk treated with sodium dodecyl sulfate, an alkyl sulfate microbicide that inactivates human immunodeficiency virus type 1. J. Hum. Lact. **22:**61–74.
24. **Heaton, J. C., and K. Jones.** 2008. Microbial contamination of fruit and vegetables and the behaviour of enteropathogens in the phyllosphere: a review. J. Appl. Microbiol. **104:**613–626.
25. **Howett, M. K., and J. P. Kuhl.** 2005. Microbicides for prevention of transmission of sexually transmitted diseases. Curr. Pharm. Des. **11:**3731–3746.
26. **Howett, M. K., et al.** 1999. A broad-spectrum microbicide with virucidal activity against sexually transmitted viruses. Antimicrob. Agents Chemother. **43:**314–321.
27. **Kapikian, A. Z., et al.** 1972. Visualization by immune electron microscopy of a 27-nm particle associated with acute infectious nonbacterial gastroenteritis. J. Virol. **10:**1075–1081.
28. **Karst, S. M., C. E. Wobus, M. Lay, J. Davidson, and H. W. I. V. Virgin.** 2003. STAT 1-dependent innate immunity to a Norwalk-like virus. Science **299:** 1575–1578.
29. **Katpally, U., C. E. Wobus, K. Dryden, H. W. I. V. Virgin, and T. J. Smith.** 2008. Structure of antibody-neutralized murine norovirus and unexpected differences from virus like particles. J. Virol. **82:**2079–2088.
30. **Koopmans, M., and E. Duizer.** 2004. Foodborne viruses: an emerging problem. Int. J. Food Microbiol. **90:**23–41.
31. **Krebs, F. C., S. R. Miller, D. Malamud, M. K. Howett, and B. Wigdahl.** 1999. Inactivation of human immunodeficiency virus type 1 by nonoxynol-9, C31G, or an alkyl sulfate, sodium dodecyl sulfate. Antiviral Res. **43:**157–173.
32. **Li, J., E. C. Fontaine-Rodriguez, and S. P. Whelan.** 2005. Amino acid residues within conserved domain VI of the vesicular stomatitis virus large polymerase protein essential for mRNA cap methyltransferase activity. J. Virol. **79:**13373–13384.

33. **Lou, F., H. Neetoo, H. Chen, and J. Li.** 2011. Inactivation of human norovirus surrogate by high pressure processing: effectiveness, mechanism, and potential application in the fresh produce industry. Appl. Environ. Microbiol. **77:**1862–1871.
34. **Lynch, M. F., R. V. Tauxe, and C. W. Hedberg.** 2009. The growing burden of foodborne outbreaks due to contaminated fresh produce: risks and opportunities. Epidemiol. Infect. **137:**307–315.
35. **Ma, Y., and J. Li.** 2011. Vesicular stomatitis virus as a vector to deliver virus-like particles of human norovirus: a new vaccine candidate against an important noncultivable virus. J. Virol. **85:**2942–2952.
36. **Mead, P. S., et al.** 1999. Food-related illness and death in the United States. Emerg. Infect. Dis. **5:**607–625.
37. **Mukherjee, A. K., and K. Das.** 2010. Microbial surfactants and their potential applications: an overview. Adv. Exp. Med. Biol. **672:**54–64.
38. **Panyukov, Y. V., M. A. Nemykh, E. N. Dobrov, and V. A. Drachev.** 2008. Surfactant-induced amorphous aggregation of tobacco mosaic virus coat protein: a physical methods approach. Macromol. Biosci. **8:**199–209.
39. **Patel, M. M., A. J. Hall, J. Vinje, and U. D. Parashar.** 2009. Noroviruses: a comprehensive review. J. Clin. Virol. **44:**1–8.
40. **Pönkä A., L. Maunula, C. H. von Bonsdorff, and O. Lyytikäinen.** 1999. An outbreak of calicivirus associated with consumption of frozen raspberries. Epidemiol. Infect. **123:**469–474.
41. **Rawsthorne, H., T. G. Phister, and L. A. Jaykus.** 2009. Development of a fluorescent in situ method for visualization of enteric viruses. Appl. Environ. Microbiol. **75:**7822–7827.
42. **Rockx, B., et al.** 2002. Natural history of human calicivirus infection: a prospective cohort study. Clin. Infect. Dis. **35:**246–253.
43. **Rzezutka, A., and N. Cook.** 2004. Survival of human enteric viruses in the environment and food. FEMS Microbiol. Rev. **28:**441–453.
44. **Seymour, I. J., and H. Appleton.** 2001. Foodborne viruses and fresh produce. J. Appl. Microbiol. **91:**759–773.
45. **Sivapalasingam, S., C. R. Friedman, L. Cohen, and R. V. Tauxe.** 2004. Fresh produce: a growing cause of outbreaks of foodborne illness in the United States, 1973 through 1997. J. Food Prot. **67:**2342–2353.
46. **Song, H., et al.** 2010. Thermal stability and inactivation of hepatitis C virus grown in cell culture. Virol. J. **7:**40–52.
47. **Teunis, P. F., et al.** 2008. Norwalk virus: how infectious is it? J. Med. Virol. **80:**1468–1476.
48. **Tree, J. A., M. R. Adams, and D. N. Lees.** 2005. Disinfection of feline calicivirus (a surrogate for Norovirus) in wastewaters. J. Appl. Microbiol. **98:**155–162.
49. **Urbanucci, A., M. Myrmel, I. Berg, C. H. von Bonsdorff, and L. Maunula.** 2009. Potential internalisation of caliciviruses in lettuce. Int. J. Food Microbiol. **135:**175–178.
50. **Urdaneta, S., et al.** 2005. Inactivation of HIV-1 in breast milk by treatment with the alkyl sulfate microbicide sodium dodecyl sulfate (SDS). Retrovirology **2:**28–38.
51. **Vandekinderen, I., F. Devileghere, B. De Meulenaer, P. Ragaert, and J. Van Camp.** 2009. Optimization and evaluation of a decontamination step with peroxyacetic acid for fresh-cut produce. Food Microbiol. **26:**882–888.
52. **Wei, J., Y. Jin, T. Sims, and K. E. Kniel.** 2010. Manure- and biosolids-resident murine norovirus 1 attachment to and internalization by Romaine lettuce. Appl. Environ. Microbiol. **76:**578–583.
53. **Wobus, C. E., L. B. Thackray, and H. W. I. V. Virgin.** 2006. Murine norovirus: a model system to study norovirus biology and pathogenesis. J. Virol. **80:**5104–5112.